



CORVINUS UNIVERSITY OF BUDAPEST

**BIOLOGICAL AND TECHNOLOGICAL ASPECTS OF
MICROPROPAGATION OF *HOSTA* VARIETIES**

Thesis of PhD dissertation

SZAFIÁN ZSOLT

Supervisor:
Erzsébet Jámbor-Benczúr

Written at the Department of Floriculture and Dendrology
Corvinus University of Budapest

Budapest
2010

PhD School

Name: Doctoral School of Horticultural Sciences

Field: Crop Sciences and Horticulture

Head of *PH.D.* School: Prof. Dr. Magdolna Tóth
Doctor of the Hungarian Academy of Sciences
Head of Department of Fruit Sciences
CORVINUS UNIVERSITY OF BUDAPEST,
Faculty of Horticultural Sciences

Supervisor: Prof. Dr. Erzsébet Jámor-Benczúr
Doctor of the Hungarian Academy of Sciences
CORVINUS UNIVERSITY OF BUDAPEST,
Faculty of Horticultural Sciences

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

.....
Head of *PH.D.* School

.....
Supervisor

1. THE ANTECEDENTS AND GOALS OF THE WORK

The laboratory of Department of Floriculture and Dendrology at the Corvinus University of Budapest is one of the first's *in vitro* propagation research laboratories in Hungary. During the last decades managed to work out technology for *in vitro* propagation of several ornamental plants. The working out the *in vitro* propagation technology of *Hosta* taxons also belong to this research project.

In Europe and in the USA this long lived, shade tolerant perennials are very popular. In every year dozens of new varieties appear on the market, and now the number of varieties more than 4000. These trends affect the Hungarian market, and made a higher demand for these perennials.

The *Hostas* traditionally propagated by division in spring, but during the last years the micropropagation become an important propagation method. The benefit of this method is to gain many plants from one plant. The disadvantage of this method is the higher price. This higher price is in Hungary only enforceable in case of new varieties during the first years. Further disadvantage is the somaclonal variability and the split of the features of varieties with chimera origin.

The goal of my research was to introduce some new *Hosta* varieties to Hungary, and working out a propagation technology for the commercial propagation. This technology covers the shoot induction and multiplication and the determination of an effective propagation medium.

I laid emphasis on the determination of the quality and quantity of the carbohydrates and growth regulators.

During my researches I wanted to

- process the disinfection method, and establish an *in vitro* culture
- determine the medium for shoot induction, for multiplication and for rooting, considering
 - o the effects of the growth regulators (benzyl-adenine, kinetin and NAA)
 - o the effect of the quality and quantity of the carbohydrates (saccharose, glucose and fructose)
- examine the possibilities of preserving the features of varieties
- test the worked out technology in a commercial laboratory

Besides the working out the propagation technology, with anatomical survey I liked to investigate the changes during the *in vitro* propagation.

With the histological research I set

- the examination of the histological structure of *ex vitro* developed plants, and comparison with the
 - anatomical changes of *in vitro* developed plants, and
 - changes during acclimatisation

2. MATERIALS AND METHODS

I started my research at the University of Horticulture and Food Industry in 1993, when I worked out a micropropagation technology for *Hosta fortunei* 'Albopicta'. With lean on the results of this research, in 1995 I started to working out new propagation technology for additional varieties. In this year I bought 40 *Hosta* taxons – which were not known in Hungary –from Marco Fransen Nursery, (Ter Are, The Netherlands). From these 40 varieties I selected 6 for my further researches. The selected varieties are.

Hosta 'Blue Cadet'

Hosta 'Devon Green'

Hosta 'Dew Drop'

Hosta 'Gold Drop'

Hosta 'Gold Haze'

Hosta 'Samurai'

2.1. Establishment of cultures

The buds of the rhizomes were used. The buds were cut of from the rhizome and two disinfection methods were tested. The method, which was used in he case of *Hosta fortunei* 'Albopicta' was effective. This method was compared with a less destructive method.

1. method:
 - soaking in 0,5 % HgCl₂ solution for 10 minutes
 - soaking in 70% ethyl-alcohol solution for 10 minutes
 - rinsing in sterile distilled water, three times

2. method.
- soaking in a doubly diluted Clorox solution (NaOCl content app. 2,5%) for 10 minutes
 - soaking in 70% ethyl-alcohol solution for 5 minutes
 - rinsing in sterile distilled water, three times

The outer 2-3 bud scales were removed, and the remains of the rhizome were cut of. The prepared buds were placed a hormone free medium. After 1 week the not infected buds were placed onto media containing 6 mg/l, 3 mg/l benzyl-adenine or 3 mg/l kinetin for multiplication. 12 buds were used per varieties per medium. During the multiplication phase the only the axillary shoots were used.

2.2. The effect of various carbohydrates on the proliferation of different varieties and the percentage of true to variety plants

During the multiplication phase the culture cycle was 6 weeks. All treatments were repeated 3 times, with 12 samples.

In the experiments the bellow listed features were investigated:

- I. The effect of the quantity of saccharose on the multiplication
- II. The effect of the different carbohydrates on the multiplication and the percentage of true to variety plants

2.2.1. The effect of the quantity of saccharose on the multiplication

For the investigation of the effect of the quantity of saccharose on the multiplication, *Hosta* 'Dew Drop', *H.* 'Gold Drop' and *H.* 'Gold Haze' cultures were used. The saccharose content of media varied between 0-40 g/l. The media contained 2 mg/l benzyl-adenine and 0,1 mg/l naftil acetic acid.

2.2.2. The effect of the different carbohydrates on the multiplication and the stability of varieties

In the experiments the effect of three carbohydrates were examined in concentration of 0-50 g/l. The media contained 2 mg/l benzyl-adenine and 0,1 mg/l naftil

acetic acid. In the experiments the green leaved *H. 'Devon Green'*, the blue leaved *H. 'Blue Cadet'* and the variegated leaved *H. 'Dew Drop'* and *H. 'Samurai'* were used. The latter two, variegated leaved variety with chimera origin are tend to lose the true to variety during *in vitro* propagation. This research aimed at to determine the influence of the quality and quantity of carbohydrates on the stability of *Hosta* varieties.

2.3. *In vitro* rooting

A propagation technology can't be complete without rooting the shoots. In the rooting phase 5 different media were tested. Beside the growth regulator free medium, media containing 0,1 mg/l NAA and 1 g/l active charcoal or 0,2 g/l potassium-humate or 20 ml/l fulvic acid were examined.

2.4. Conditions of *in vitro* propagation

My researches were done at the laboratory of Department of Floriculture and Dendrology of former University of Horticulture and Food Industry (today Corvinus University of Budapest). The commercial propagation was done at the Biotechnological laboratory of the Erdészeti Rt. of Szombathely. My result about the commercial propagation originated there.

In the initiation phase 100 ml Erlenmeyer flask were used. During the autoclaving of media the flasks were covered with aluminium foil, the jars were covered with aluminium foil and with its lid. Multiplication was carried out in 220-ml jars containing 40 ml of medium and closed with clear plastic film. For the commercial propagation Vegbox were used. Plants were cultured on top-illuminated growth shelves on $22\pm 2^{\circ}\text{C}$ temperature, 16/8 hours photoperiod, with $20\ \mu\text{M}/\text{m}^2/\text{s}$ light intensity.

2.5. Condition of acclimatization and outdoor growing

The most of rooted shoots were acclimatized in a greenhouse, under plastic tunnel with bottom heating. A small number of rooted cutting were acclimatized under shaded plastic tunnel.

For the acclimatization the rooted shoots were planted to multicell-trays (104 holes), into a mixture of Baltic peat and perlite in 2:1 rate. The acclimatize plants were transplanted to 5.5 cm plastic pots. The final pots were 10 cm in diameter. The substrate was a mixture of Baltic peat, black peat and alginite in 6:3:1 rate. For fertilization

4,5 kg/m³ Osmocote Exact (8-9 m) control released fertilizer and 1,5 g/l Kristalon water soluble fertilizer were used.

In case of commercial propagation the rooted shoots were planted to Jiffy pots, and were acclimatized in a greenhouse. The acclimatized plants were cultivated in a greenhouse for a month in the Prenor Nursery, than were transplant to 8x8x8 cm square pots. In the second year the plants were transplanted into 2 litre pots. The growing substrate was Stender MC 530 type substrate (60% Baltic peat, 30% black peat and 10% clay). For fertilization 4,5 kg/m³ Osmocote Exact (8-9 m) control released fertilizer was used.

2.6. Plant characteristics investigated

Development of the plants was evaluated following a 6 weeks culture cycle. Growth parameters like as the number of the shoots and roots, length of the shoots and roots, length and width of leaves were measured. The uniformity of cultures and the percentage of true to variety plants were examined.

2.7. The methods of evaluation of histological examination

For the histological examinations light microscope and scanning electron microscope were used. For the investigation of leaf anatomy samples were collected from the *in vitro* grown, from the acclimatized and from outdoor grown plants. The samples were taken from the 3rd youngest leaf of shoots. During the multiplication phase the samples were collected from plants grown on the same media, containing 2 mg/l BA. Samples were collected from the acclimatizing plants and from the already acclimatized, but greenhouse growing plants.

The light- and scanning electron microscopic investigations were done in the Central Laboratory of University of Horticulture and Food Industry.

For the light microscopic investigations the samples were stained with toluidine blue and embedded in epoxy-resin and thin sections were cut with microtome. Photos were made with Canon camera.

For the electron microscopic investigations the samples were dehydrated and covered with a thin gold layer. For the investigation Tesla BS 300 scanning electron microscope were used.

2.8. Statistical evaluation of the data

Every treatment was repeated three times, and twelve plants were investigated in one repetition. Statgaf 3.0 and Excel 5.0 computer program and variance analysis were used for the evaluation of data. The results were represents on charts.

3. RESULTS

I present the results of my research related to the phases of the micropropagation technology.

3.1. Establishment of culture, disinfection methods

The effect of HgCl_2 solution (I. method) was excellent; the rate of uninfected inoculums was more than 85%. The effect of Clorox solution (II. method) was a bit less effective, the rate of uninfected inoculums was 70%. The disinfection of plants in any case causes a stress. Because of it, the after-effect of the disinfection was evaluated on the 3 rd. week after the start of culture. The Clorox solution has less damaging after-effects, and the growths of shoots were faster and much more powerful.

The most shoots in case *H.* 'Blue Cadet', *H.* 'Devon Green', and *H.* 'Samurai' developed on media containing 6 mg/l BA, while in case of *H.* 'Dew Drop', *H.* 'Gold Drop' and *H.* 'Gold Haze' developed on media containing 3 mg/l BA. The application of kinetin has worse results on all variety than BA in any applied concentration.

During the multiplication phase the multiplication rate was measured. After the 6th subculture the results showed that *H.* 'Dew Drop', *H.* 'Gold Drop' had a high multiplication rate. They had significantly higher multiplication rate than the other varieties. The multiplication rate of *H.* 'Gold Haze', *H.* 'Devon Green' and *H.* 'Blue Cadet' significantly lower than the above mentioned two varieties, and significantly higher than in case of *H.* 'Samurai'. The *H.* 'Samurai' has the significantly lowest multiplication rate. Although the multiplication rates are different, the varieties with closer relationship have similar results.

3.2. The effect of different carbohydrates on the multiplication and the stability of varieties

3.2.1. Effect of quantity of saccharose on multiplication

I wanted to determine, if the saccharose concentration has any influence on the multiplication rate of *Hosta* taxons.

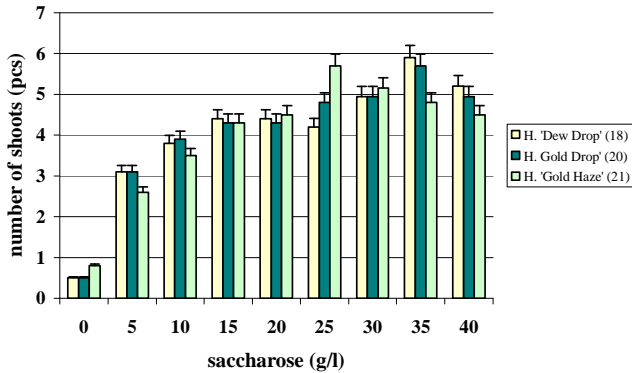


Figure 1. Number of shoots on media containing different amount of saccharose

The 1. figure shows the number of shoots of three *Hosta* varieties. On the saccharose free media both three variety grown poorly, the average multiplication rate was less than 1. In case of *H. 'Dew Drop'* and *H. 'Gold Drop'* the media containing 35 g/l saccharose achieved the best results, while in case of *H. 'Gold Haze'* media containing 25 g/l saccharose was the optimal.

From the results excels, that the amount of saccharose has an essential effect on the growth of cultures. In case of *H. 'Dew Drop'* it is conspicuous; because of it has an effect on the rate of variegation. It is easily can be seen if the plants are true-to-variety or not (Fig.2).



Figure 2. Cultures of *Hosta* 'Dew Drop' developed on 15 g/l saccharose (left) and 35 g/l saccharose (right) containing media

3.2.2. *Effect of different carbohydrates on the multiplication and the stability of varieties*

Referring to references, in case of some plant the application of fructose or glucose can give better results than saccharose. To determine the effects of saccharose, fructose and glucose on the multiplication rate and on stability a comparative experiment were set with *H. 'Devon Green'*, *H. 'Blue Cadet'*, *H. 'Dew Drop'* and *H. 'Samurai'*. In the experiments plantlets were placed onto medium containing 0, 5, 10, 20, 30, 40, 50 g/l saccharose, fructose or glucose.

The number of developed shoots was very different by varieties. The most shoots of 'Blue Cadet', 'Dew Drop' and 'Samurai' developed on saccharose containing media, in case of 'Devon Green' both on fructose and glucose containing media developed more shoots.

The most shoots of *H. 'Devon Green'* had developed on media containing 10 g/l fructose (5,6 pcs.), and among the fructose containing medium the on the same concentration was the most effective (4,8 shoots). This variety is very sensitive to high carbohydrate concentration. On higher concentration the number of shoots was quickly decreased.

The most shoot of *H. 'Blue Cadet'* had developed on 20 g/l saccharose containing media. From the fructose or glucose containing medium on the same, 20 g/l concentration had developed the most shoots. On the saccharose containing media developed 7,2 shoots is significantly more than on fructose containing media developed 5,2 shoots or than on glucose containing media developed 5,6 shoots. The effect of fructose and glucose are not significantly different.

In case of *H. 'Dew Drop'* the most shoots (5,2 pcs) had developed on the 40 g/l saccharose containing media. Among the fructose or glucose containing media in both case the 20 g/l concentration was the best (4,8 pcs).

The tendency in case of *H. 'Samurai'* was similar. The most shoots had developed on 30 g/l saccharose containing media (4,8 pcs.). The optimal concentration of fructose or glucose was 20 g/l (4,2 pcs. resp. 4,3 pcs.).

The uniformity of cultures of *H. 'Dew Drop'* was different on fructose, glucose or saccharose containing media. On glucose and saccharose containing media the edge of the leaves was white, on fructose containing media it had become yellowish. By the increasing carbohydrate concentration the markings of leaves had become stronger; the white margin

had become wider (Fig. 3.). The cultures had become more uniform, the number of true-to-variety plantlets had increased, and the number of totally green plantlets had decreased.



Figure 3. Leaves of *Hosta* 'Dew Drop' (developed on 10, 15, 25 and 50 g/l saccharose containing media)

In case of *H. 'Samurai'* the markings of the leaves had become stronger on higher carbohydrate concentration, especially on higher saccharose concentration. The selection of true-to-variety plantlets was difficult, because of the yellow leaf margin had become greenish, and become hard distinctive. Because of it, the distinction of true-to-variety and all yellow plantlets can be made only after acclimatization.

3.3. The effect of plant growth regulators and other natural supplements on the rooting of *Hosta* taxons

The shoots developed in the multiplication stage were placed onto rooting media. The media contained NES, active charcoal, potassium-humate or fulvic-acid.

The results showed that the shoots had rooted on every tested media, but the quality of developed roots was different. The NES made the roots shorter and thicker. On the medium containing active charcoal the appearance of roots was faster, but the roots were thinner and weaker. The application of NES and potassium humate resulted better root quality, but the effect of fulvic acid was adverse.

3.4. Histological changes during the multiplication and rooting of *Hosta* taxons

The effects of micropropagation were investigation in several varieties.

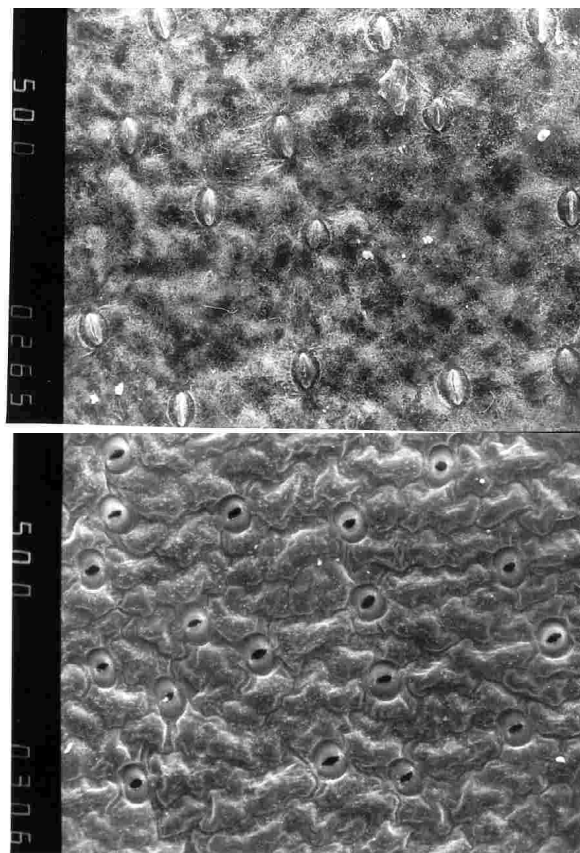


Figure 4. Abaxial epidermis of outdoor (up) and *in vitro* (down) developed leaves of *H. 'Samurai'* (SEM, 500x magnification).

By the microscopic shootings has been found the cells of upper epidermis of leaf during the *in vitro* propagation lost their roundish forms and the walls become more undulate. The cells become more raised, and the walls not suit as close as in the open field grown plants. The leaves had a less developed cuticle, and the wax layer is very thin or missing. The leaves of *Hosta* plantlets have stomata distributed on both faces of the epidermis, however, with greater number on the lower face. The cells of abaxial epidermis are smaller, and have more undulate wall. The size of stomata different in *in vitro* and *ex vitro* developed leaves. The stomata of *in vitro* developed leaves a slightly bigger than *ex vitro* developed and usually opens (Fig. 4). The thin mesophyll presents disorganized

spongy and palisade parenchyma with large intercellular spaces. The rate of spongy parenchyma is higher than in open field grown leaves.

During the acclimatization the stomatal density decreases. At the time of transplant to ex vitro conditions the plants have *in vitro* developed stomata, which hardly react to ex vitro conditions. The closing of stomata is a long process. On the 3rd day after transplanting, only the 50 % of stomata closed. The first ex vitro developed leaves appearing on the 10 day after transplanting. They have effective stomatal regulation, but the stomatal density is higher than in ex vitro developed leaves.

3.5. The result of the commercial propagation

The mass propagation of *Hosta* varieties takes place in the Biotechnical Laboratory of Szombathelyi Erdészeti Rt. since 1998. The new technology is appropriate for commercial production. For the acclimatization the best time is between April and May. The later acclimatized plants have not enough time to root well till the winter frosts. The well rooted potted plans are transplanted during spring to 2 litres containers, and grow to sellable plants in the second part of the year.

3.7. New scientific results

1. On the basis of my experiments an effective disinfection method was worked out for 13 *Hosta* varieties, with which the stress caused by disinfection can be reduced, and faster development can be achieved.

2. The composition of media used in the initial phase had been determined for the 13 varieties involved in experiments.

3. In the proliferation phase the optimal concentration of cytokinines was quantified, which ensure with great efficiency the developing of shoots in good quality.

4. The concentration of carbohydrates used in proliferation phases of micropropagation was quantified.

5. The compounds of effective media used in rooting phase were determined.

6. The results of comparative histological study of *in vitro* grown leaves gave new information about the anatomical effects of micropropagation.

7. The implemented sterile cultures of the 13 examined varieties are suitable for mass propagation. Based on my results the commercial propagation of the 6 varieties mentioned in this dissertation, and 3 other varieties had been realized. The plants propagated

by this technology have been on the nursery market for years. The micropropagation technology established by the results of this dissertation has contributed to mass propagation of further *Hosta* varieties.

4. CONCLUSIONS AND SUGGESTIONS

4.1. General conclusions

- The use of buds of rhizome has proved to be sufficient for all investigated varieties. In this method neither callus induction nor shoot regeneration is utilized which would otherwise lead to splitting of varieties with chimera origin.

- The quantity and type of carbohydrate added to the media has great effect on the development and stability of cultures.

- There are varieties which require more than 20 g/l saccharose suggested in the literature. For the optimal development they required 30-35 g/l saccharose.

4.2. Suggested technology for *in vitro* propagation of different *Hosta* taxons

The results of my experiments are summed up in a scheme of propagation:

1. Choosing the mother plants

Before starting the culture, the selected, well growing, healthy and pathogen free plants must be observed in the whole vegetation period. It has to be ensured that the plants show every feature of the variety in every fenological phase. The mother plants must be kept outdoors until spring to get jarovized naturally.

2. Starting the culture

All soil must be washed off of the rhizome, and the roots must be cut off from the rhizome. The rhizome must be scrubbed, then washed in running water for an hour. The buds must be cut off from the rhizome so, that a small part of the rhizome remains on it. The two-three outer bud scales must be removed from the buds. The buds prepared in this way should be put into a beaker. Then a few drops of Tween 80 must be added to the buds, and after the beaker had been covered with gauze, the buds have to be washed for an hour in running water. For disinfection of the buds the following method is suggested:

- soaking in a doubly diluted Clorox solution (NaOCl content app. 2.5%) for 10 minutes
- soaking in 70% ethyl-alcohol solution for 5 minutes
- rinsing in sterile distilled water, three times

The disinfected buds must be put to hormone free MS media. The sterile buds must be transferred to the initialization media after 1 week.

The compounds of basic media are:

- MS macro elements in half concentration
- MS microelements
- MS vitamins in double concentrations, thiamine in quadrupled concentration
- adenine-sulphate: 80 mg/l; myo-inositol: 100 mg/l

The compounds of initiation media:

- Basic media
- 3 mg/l BA and 0.1 mg/l NAA for *H. 'Dew Drop'*, *H. 'Gold Drop'* and *H. 'Gold Haze'*
- 6 mg/l BA and 0.1 mg/l NAA for *H. 'Devon Green'*, *'Blue Cadet'* and *H. 'Samurai'*

3. Multiplication phase

The subculture frequency: 4-5 weeks

The compound of multiplication media:

- basic media
- 2 mg/l BA and 0.1 mg/l NAA

The suggested types and quantities of carbohydrate are listed in Table 1.

Table 1. Suggested carbohydrates for multiplication of *Hosta* taxa

| Variety | Carbohydrate | Suggested quantity (g/l) |
|-------------|--------------|--------------------------|
| Blue Cadet | saccharose | 20 |
| Devon Green | fructose | 10 |
| Dew Drop | saccharose | 35 |
| Gold Drop | saccharose | 35 |
| Gold Haze | saccharose | 25 |
| Samurai | saccharose | 30 |

4. Rooting phase

The rooting of the examined varieties was trouble free on all tested medium. In virtue of the quality of developed roots the composition of the suggested rooting medium is:

- Basic media
- 0.1 mg/l NAA and 0.2 g/l potassium-humate

5. Acclimatization

The acclimatization of rooted shoots using the generally applied methods is trouble free and takes 12-20 days, depending on the starting time.

6. Growing plants

There are usually no problems during the growing of acclimatized plants. Plants acclimatized after June have no time to root well and grow strong before the winter frosts. Therefore the acclimatization must be started at the very latest in the second half of May. The plants grow well in any peat based growing substrate, at pH 6-7. I suggest adding 4.5 kg/m³ controlled release fertilizer in to the growing substrate.

5. PUBLICATIONS IN RELATION TO THE PHD THESIS

Publications without IF:

Szafián, Zs., Jámbor-Benczúr, E., Ferenczy, A. 1995. *In vitro* propagation of *Hosta fortunei* I. Effect of growth regulators on proliferation Horticultural Science 27. 1-2: 19-22.

Szafián, Zs., Jámbor-Benczúr, E., Ferenczy, A. 1996. *In vitro* propagation of *Hosta fortunei*. II. Rooting and acclimatisation. Horticultural Science 28. 8-10.

Mándy A., Stefanovics-Bányai É., Imre Cs., **Szafián, Zs.** 2001. Results in the determination of some *Hosta* varieties by the method of isoelectric focusing Int. J. of Hort. Sci. 7 (1): 90-92.

Jámbor-Benczúr, E., Mándy, A., Sinkó, Z., Hipszki, B., **Szafián, Zs.,** Dobránszki, J. 2004. The effect of different carbohydrates on multiplication of *Hosta* cultivars Int. J. of Hort. Sci. 10 (4) 115-117.

Conference papers (Hungarian, abstracts):

Szafián, Zs. 1995 *In vitro* propagation and investigation of epidermis of *Hosta fortunei* 'Albopicta'. Abstract of price winning papers presented at the 22nd National Conference of Student's Circles. Agriculture Vol. 2. p. 22 Keszthely

Szafián, Zs., Jámborné Benczúr, E., Ferenczy, A. 1996. A *Hosta fortunei* mikroszaporítása és levél epidermiszének vizsgálata. III. Növénytermesztési Tudományos Napok, Budapest, 1996. január 22-23. p.52.

Szafián, Zs., Jámbor-Benczúr, E., Ferenczy, A. 1996. A *Hosta fortunei* mikroszaporítása A Lippay János tudományos ülésszak előadásainak és poszttereinek összefoglalói. Kertészeti és Élelmiszeripari Egyetem, Budapest. 88-89.

Mándy A., **Szafián Zs.,** Jámborné Benczúr E. 1998. Eredmények egyes *Hosta* fajták akklimatizálásában és variabilitás vizsgálatában. Lippay János - Vas Károly Nemzetközi Tudományos Ülésszak Előadási és Poszterei, Dísnövénytermesztési Szekció 98-99.

Szafián Zs., Jámborné Benczúr E., Tillyné Mándy A. 1998. A szacharóz hatása a különböző *Hosta* fajták szaporodására és klorofill tartalmára. Lippay János - Vas Károly Nemzetközi Tudományos Ülésszak Előadási és Poszterei Dísnövénytermesztési Szekció 110-111.

Jámborné Benczúr E., Hipszki B., Mándy A., Sinkó Z., **Szafián Zs.** 2000. Különböző szénhidrátok hatása a *Hosta tokudama* fajtaak *in vitro* szaporítása során. Lippay János - Vas Károly Tudományos Ülésszak november 6-7. Budapest. Összefoglalók 114-115.

Mándy A., Stefanovits-Bányai É., **Szafián Zs.** 2000: Egyes *Hosta* fajták stressztolerancia vizsgálata az akklimatizálás során. Lippay János - Vas Károly Tudományos Ülésszak november 6-7. Budapest. Összefoglalók 120-121.

Tilly-Mándy A., Jámborné Benczúr E., Császár O., Ördögh M., **Szafián Zs.,** Stefanovits-Bányai É. 2007. Eredmények a Titavit *in vitro* használatával *Hosta* 'Dew Drop' és *Hosta* 'Gold Drop' tesztnövény esetén. XIII. Növénytermesztési Tudományos Napok MTA Budapest, március 12. Összefoglalók. 121.

Ördögh M., Jámborné Benczúr E., Tillyné Mándy A., Lelik L., **Szafián Zs.** 2007. Különféle növekedésszabályozó anyagok hatásának vizsgálata *Hosta* 'Gold Drop' *in vitro* felszaporításánál. Lippay János-Ormos Imre-Vass Károly Tudományos Ülésszak 2007. november 7-8. Budapest. Összefoglalók. 70-71.

Tillyné Mándy A., Császár O., Ördögh M., **Szafián Zs.**, Jámborné Benczúr E., Stefanovits-Bányai É. 2007. A Titavit hatása *Hosta* 'Dew Drop' *in vitro* tenyésztetére. Lippay János-Ormos Imre-Vass Károly Tudományos Ülésszak 2007. november 7-8. Budapest. Összefoglalók. 88-89.

International conference papers (full paper):

Szafián, Zs., Jámbor-Benczúr, E., Csillag, F. 1997. *In vitro* propagation and study of the epidermis of *Hosta fortunei* 'Albopicta' Proceedings of the Third International Symposium on *in vitro* Culture and Horticultural Breeding Jerusalem, Izrael. Acta Horticult. 447. 191-192.

International conference papers (abstract):

Szafián, Zs., Jámbor-Benczúr, E., Csillag, F. 1996. *In vitro* propagation and study of the epidermis of *Hosta fortunei* 'Albopicta' Third International Symposium on *in vitro* Culture and Horticultural Breeding Jerusalem, Izrael. Program and Book of Abstracts 23.

Szafián, Zs., Jámbor-Benczúr, E., Tilly-Mándy, A., Komiszár L. 1998. Influence of the carbohydrate concentration on shoot production of different *Hosta* cultivars IX International Congress On Plant Tissue And Cell Culture, Jerusalem Book of Abstracts 111.

Jámbor-Benczúr, E., Mándy, A., Sinkó, Z., Dobránszki, J., Hipszky, B., **Szafián, Zs.** 2000. The effect of different carbohydrates on multiplication of *Hosta tokudama* cultivars COST 843 WGI: Developmental Biology of Regeneration, 1st Meeting, 12-15 Oct 2000, Geisenheim, Germany, 33-34

Stefanovits-Bányai É., A. Mándy, Cs. Imre, **Zs. Szafián**: Isoelectric Focusing, as a Method in the Determination of some *Hosta* Varieties. Plant Biology and Biochemistry. 12th Congress of the Federation of European Societies of Plant Physiology 2000, Budapest. p. 18.

Tilly-Mándy, A., **Szafián Zs.**, Jámbor-Benczúr, E., Ördögh, M., Stefanovits-Bányai É. 2007. The effect of titavit on the *in vitro* development of *Hosta* 'Dew Drop' and 'Gold Drop' International Scientific Conference Propagation of Ornamental Plants. 4-6. September. Sofia, Bulgaria. Book of Abstracts. 175.

Book, part of a book

Tillyné Mándy A., **Szafián Zs.** 2005 Árnyékliliomok in Jámborné Benczúr E.-Dobránszki J. (szerk.) Kertészeti növények mikroszaporítása, Mezőgazda Kiadó pp.228-229.